Heterogeneity of Soybean Trypsin Inhibitors. II. Heat Inactivation

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ABSTRACT

The five DEAE chromatographic fractions (II, III, IV_1, IV_2, V) of soybean trypsin inhibitor were less stable in heated alkaline solutions than in acid solutions. Cystine-poor fractions II and III were the most heat-stable inhibitors in acid solutions, while cystine-rich fractions IV_1 and IV_2 possessed an intermediate stability. Cystine-moderate fraction V, which was identified as Kunitz-inhibitor and $SBTIA_2$, was found to be the most heat-unstable inhibitor.

Soybean protein is a high-quality protein for human needs, but its trypsin inhibitors (TIs) and hemaglutinin (1) cause its poor utilization. It is well established that various heat treatments increase its utilization by inactivating the TIs (1,2,3,4). Kunitz (5) studied heat-inactivation of crystalline inhibitor (6)—the major soybean trypsin inhibitor (SBTI), corresponding to $SBTIA_2$ of Rackis and Anderson (7,8,9)—and found that the inhibitor was rather unstable in an alkaline solution (5). Rackis (2) reported that TI activity of the four chromatographic fractions ($SBTIA_1$, A_2, B_1, and B_2) was destroyed by stream heat. Birk (10), and Birk and Gertler (3) studied heat inactivation of another SBTI, and found that the acetone-insoluble TI was more heat stable than the Kunitz inhibitor.

We reported previously (11) that SBTI was highly heterogeneous, and found that 10 electrophoretic bands possessed TI activity, and that 7 active TI fractions were obtained by DEAE-cellulose chromatography. This paper reports differences in susceptibility to heat inactivation of the 5 DEAE chromatographic fractions (II, III, IV_1, IV_2, and V). The heat-inactivation of $SBTIA_1$, $SBTIA_2$, $SBTIB_1$, and $SBTIB_2$ of Rackis and Anderson (7,8,9) was also studied for comparison.

EXPERIMENTAL

Materials

A commercial crystalline SBTI which corresponds to the Kunitz-inhibitor (5,6) and $SBTIA_2$ of Rackis and Anderson (7,8,9) was obtained from Nutritional Biochemical Corp., Cleveland, Ohio. Samples of $SBTIB_1$, $SBTIB_2$, and $SBTIA_1$ of Rackis and Anderson (8) were kindly supplied by A. C. Eldridge, Northern Regional Research Laboratory, U.S. Department of Agriculture, Peoria, Ill.

SBTI fractions II, III, IV_1, IV_2, and V were prepared from water-extractable soybean protein (12) by DEAE-cellulose column chromatography, as described previously (11).

Estimation of Trypsin and Trypsin-Inhibitor Activities

Trypsin and TI activities were determined by the casein-digestion method of Kunitz (5). The substrate was 1% casein solution in 0.1M potassium phosphate buffer at pH 7.6. The reaction was performed at 35°C. for 20 min., 5%

trichloroacetic acid was added, and absorbancy of the filtrate at 280 nm. was determined. Trypsin (twice crystallized and salt-free) was obtained from Nutritional Biochemical Corp., Cleveland, Ohio. The heat inactivation was performed by the procedures of Kunitz (5); inactivation was expressed as percentage of inhibiting activity.

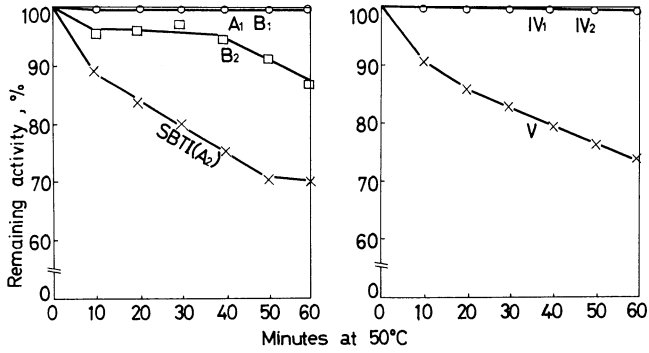

Fig. 1. Inactivation of trypsin inhibitors in 0.0025M HCl at 50°C.

RESULTS

Heating in 0.0025M HCl

Figure 1 shows the inactivation of the TIs at 50°C. The commercial TI which corresponds to $SBTIA_2$ of Rackis and Anderson was more easily inactivated than other inhibitors of Rackis and Anderson ($SBTIB_1$, $SBTIB_2$, $SBTIA_1$). In 1 hr. at 50°C., $SBTIA_2$ retained 70% of the original inhibitory activity and $SBTIB_2$ retained 88%, whereas $SBTIA_1$ and $SBTIB_1$ retained more than 99% of the original activities. The chromatographic fractions IV_1, IV_2, and V were examined under the identical conditions. Fraction V behaved exactly as $SBTIA_2$, whereas IV_1 and IV_2 retained 98% of the original activity in 1 hr. at 50°C. When the temperature was increased from 50° to 70°C. (Fig. 2), $SBTIA_2$ inactivated very rapidly during the

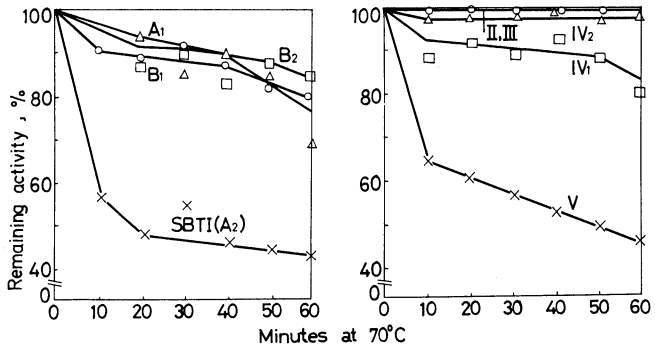

Fig. 2. Inactivation of trypsin inhibitors in 0.0025M HCl at 70°C.

first 10 min., and retained 45% of the original activity in 1 hr. Fraction V behaved similarly with $SBTIA_2$. Both IV_1 and IV_2 were inactivated slightly, whereas II and III were unaffected by the heat treatment. $SBTIB_1$, $SBTIB_2$, and $SBTIA_1$ behaved similarly to fraction IV_1.

Heating in 0.1M HCl

The heat inactivation of various TIs in 0.1M HCl at 36° and 70°C. are shown in Figs. 3 and 4, respectively. At 36°C., $SBTIA_2$ was inactivated significantly and lost

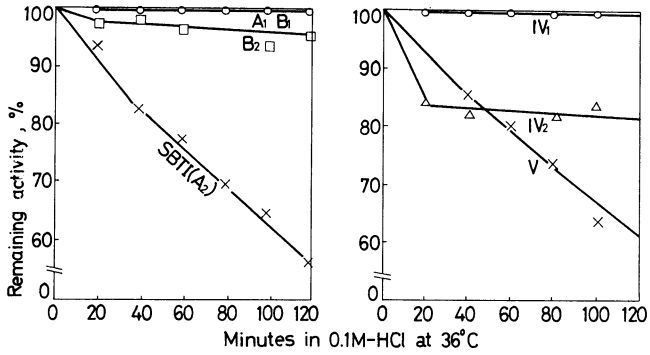

Fig. 3. Inactivation of trypsin inhibitors in 0.1M HCl at 36°C.

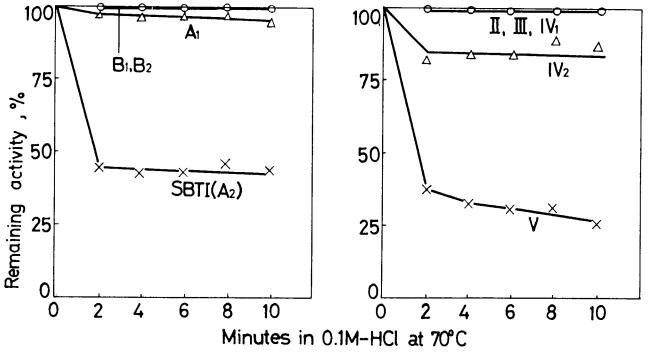

Fig. 4. Inactivation of trypsin inhibitors in 0.1M HCl at 70°C.

about 50% of the inhibitory activity in 2 hr. At 70°C., $SBTIA_2$ lost more than 50% of the activity in 2 min., but it maintained its activity for additional 8 min. at the same level. $SBTIB_1$, $SBTIB_2$, and $SBTIA_1$ were rather stable in the acid solution and retained more than 95% of the original activity at 36°C. in 2 hr. or at 70°C. in 10 min. Fraction V behaved identically with $SBTIA_2$ at both 36° and 70°C. Fraction IV_2 was relatively unstable as compared to fractions II, III, and IV_1.

Heat Inactivation in 0.1M NaOH

Figures 5 and 6 show the heat inactivation of various TIs of 0.1M NaOH at 36° and 70°C., respectively. At 36°C., $SBTIA_2$ and V were most easily inactivated, and

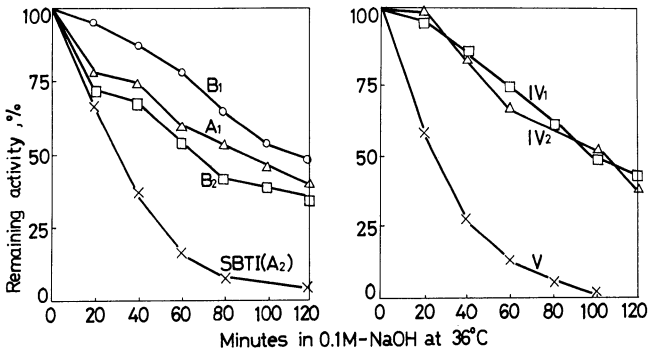

Fig. 5. Inactivation of trypsin inhibitors in 0.1M NaOH at 36°C.

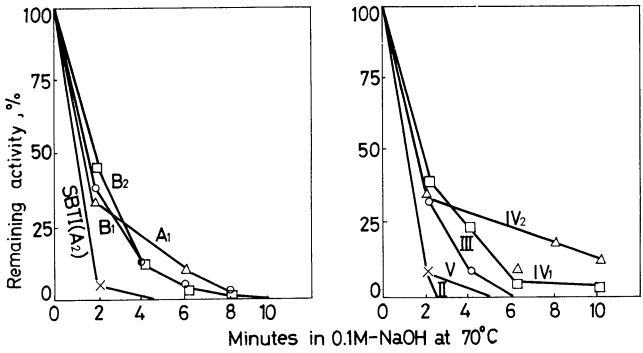

Fig. 6. Inactivation of trypsin inhibitors in 0.1M NaOH at 70°C.

both samples were almost completely inactivated in 2 hr. $SBTIB_2$, $SBTIA_1$, IV_1, and IV_2 behaved similar to each other and lost half of the activity in 2 hr. $SBTIB_1$ was more stable than $SBTIB_2$ and $SBTIA_1$. At 70°C., all fractions lost half of the activity in 2 min. and almost all of the activity in 10 min. Fractions II and III, which were very stable in the acid solutions, were very unstable in the alkaline solution.

DISCUSSION

The present results clearly indicate that the various SBTI fractions have different susceptibility to heat inactivation. Fraction V was the most unstable TI and behaved identically with the commercial TI. Since the commercial crystalline trypsin inhibitor corresponded to the Kunitz-inhibitor and $SBTIA_2$ of Rackis and Anderson (8,9), the identity of heat stability of fraction V with the commercial crystalline sample is additional proof that fraction V is identical to Kunitz-inhibitor and $SBTIA_2$.

Birk (10,13) and Aso (4) reported that the acetone-insoluble inhibitors were cystine-rich inhibitors, and Birk (13) found that the purified acetone-insoluble inhibitor (Inhibitor AA) was much more heat stable than Kunitz-inhibitor. Both fractions IV_1 and IV_2 have more than 8% of cystine and they are also cystine-rich

inhibitors (11). These fractions had an intermediate heat stability and were also more stable than fraction V. The heat stability of fractions IV_1 and IV_2 was similar to that of $SBTIB_2$ and $SBTIA_1$ of Rackis and Anderson, but not identical. It was shown previously (11) that fraction IV_2 was still heterogeneous, and the minor component(s) still associated in the major inhibitors in these fractions might have caused these differences.

Fractions II and III were cystine-poor inhibitors and still highly heterogeneous (11). Both fractions are very stable in the acid solution. $SBTIB_1$, which corresponded to the major component of fraction III (11), was also stable in the acid solution.

Though these inhibitor fractions showed different heat stabilities under various acidic conditions, they were almost equally unstable at 70°C. in 0.1M NaOH. It has been shown that the disulfide bonds in the TIs play an important role in their stability (13,14), and that one of the two disulfide bonds in Kunitz-inhibitor is essential for the activity (14). Hydrolysis of disulfide bonds in the heated alkaline solution may be responsible for the instability of these inhibitor fractions.

Acknowledgments

This research has been financed in part by a grant made by the U.S. Department of Agriculture under P.L. 480. The authors thank A. C. Eldridge for samples of $SBTIB_1$, $SBTIB_2$, and $SBTIA_1$.

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[Received June 8, 1970. Accepted March 26, 1971]